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INVESTIGATING ECTOPARASITE DIVERSITY IN ENDEMIC FISHES : *PUNTIUS* SP. AND *ANABAS* SP. FARMED IN INDONESIA

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ABSTRACT

This study aims to determine the variety of ectoparasites in *Puntius* sp. and *Anabas* sp. fish from aquaculture activities. A total of 60 *Puntius* fish and 60 *Anabas* fish were taken for examination of ectoparasites on the gills and body surface. A total of 4 ectoparasites were found to infest *Puntius* fish, namely *Trichodina* (78.3% in prevalence), *Ichthyopthirius multifiliis* (10% in prevalence), *Dactylogyrus* (50% in prevalence), and *Gyrodactylus* (15% in prevalence); while 5 ectoparasites were found to infest *Anabas* fish, namely *Ichthyopthirius multifiliis* (8.3% in prevalence), *Trichodina* (61% in prevalence), *Dactylogyrus* (45% in prevalence), *Argulus japonicus* (5% in prevalence), and *Lernaea cyprinacea* (1.6% in prevalence). *Trichodina* is the parasite found in greatest abundance in both endemic fishes.There were differences in the number, type and prevalence of ectoparasites infesting the two endemic farmed fish. *Trichodina* was found with the highest prevalence in both endemic fish species farmed in Indonesia. This is the first finding to provide an understanding of the types and patterns of ectoparasite infestation in the two species of wader farmed in Indonesia.

Keywords: aquaculture. diversity. endemic fish. ectoparasite. fish health

INTRODUCTION

Indonesia has a variety of endemic fish species that have the potential to be cultivated as a source of food through fish farming activities. *Puntius* and *Anabas* are examples of such endemic fishes that have been successfully cultivated for consumption. The endemic fish farming activities in Indonesia are constantly being improved as an effort to domesticate, fulfill the needs of food ingredients and improve the economy of local communities.

Puntius sp. is an endemic fish in Indonesia distributed in Kalimantan, Sumatra, Sulawesi, and Java with various local names. Meanwhile, *Anabas* sp. is found in Java Island with the local name Betok fish. Both of these fish can be found in waters in Indonesia, such as rivers and swamps. Up to 2023, *Puntius* and *Anabas* aquaculture activities in Indonesia have been developing with various cultivation technologies [1, 2, 3].

The obstacle that is still found in *Puntius* and *Anabas* fish aquaculture activities is the infestation of ectoparasites. The presence of ectoparasites can cause fish to reduce appetite, then gradually weaken and lead to mortality [4]. Another loss can be skin and gill damage, slow growth, reduced selling value and rejected by consumers due to abnormal fish shape [5] Several reports regarding ectoparasites in *Puntius* [6, 7, 8] and *Anabas* [9, 10] have been reported separately. This underlies the need for a study that examines parasite infestation in two endemic fish considering that parasite infestation patterns are not always the same due to the influence of various factors, such as water quality conditions, fish condition, and pathogenic organisms [11]. This study is the first to report in investigating the diversity of ectoparasites in two farmed Indonesian endemic fish species. This finding can be used as study material for ectoparasite prevention in aquaculture of endemic fish to increase their production value and developing sustainable fisheries management strategies that consider biological and ecological factors of parasite infestation in both endemic fish.

MATERIAL AND METHODS

Sample collection

A total of 60 *Puntius* and 60 *Anabas* were collected from local fish farmers in Pasuruan, East Java, Indonesia from September 2023 to February 2024. This location was chosen due to the active farming activities of these two endemic fishes. *Puntius* fish collected were 6-10 cm in size, while *Anabas* fish collected were 9-12 cm in size. *Puntius* and *Anabas* fish at the study site were reared in concrete and earthen ponds. The selection of ponds due to the existence of active *Puntius* and *Anabas* in farming activities, also the water source of these farming activities originates from groundwater sources and rivers. Based on observations at the location and information from local farmers, the water source used is not through the treatment process so it is thought to be a supporting factor for the emergence of parasites. The selected ponds are those managed by independent parties (local farmers) and the government. Prior to sampling, a survey was conducted to the pond owners to convey the purpose of the study and its benefits.

Puntius and *Anabas* fish samples were collected randomly using a fishing net and then put into a plastic bag and provided with oxygen. Fish samples were then carried to the laboratory for parasite examination.

Ectoparasite examination

The examination of ectoparasites was carried out by scrapping the body surface and gills and observed under a binocular microscope (OPTIKA B-192, Italy), binocular microscope (Nikon-E E-200 with Camera Set) with 100x and 400x magnification [12, 13, 14]. The scrapped result was placed in an object glass and then dripped with distilled water. After that, the scrapped result was covered with a cover glass. Parasite identification refers to [15] and [16] for *Trichodina* examination, [17] and [18] for *Ichthyopthirius multifiliis* examination, [19] and [20] for *Dactylogyrus* examination, [21] and [22] for *Argulus* examination, [23] for *Gyrodactylus* examination, [24] for *Lernaea* examination.

Data Analysis

Total parasites and their average were calculated to determine the number of parasites in each fish. The data was visualized through graphic and table. Prevalence and intensity calculation is based on [25] and [26] formulated as follows :

$$\text{Prevalence} = \frac{\text{Number of parasitized fish}}{\text{Number of fish examined}} \times 100\%$$

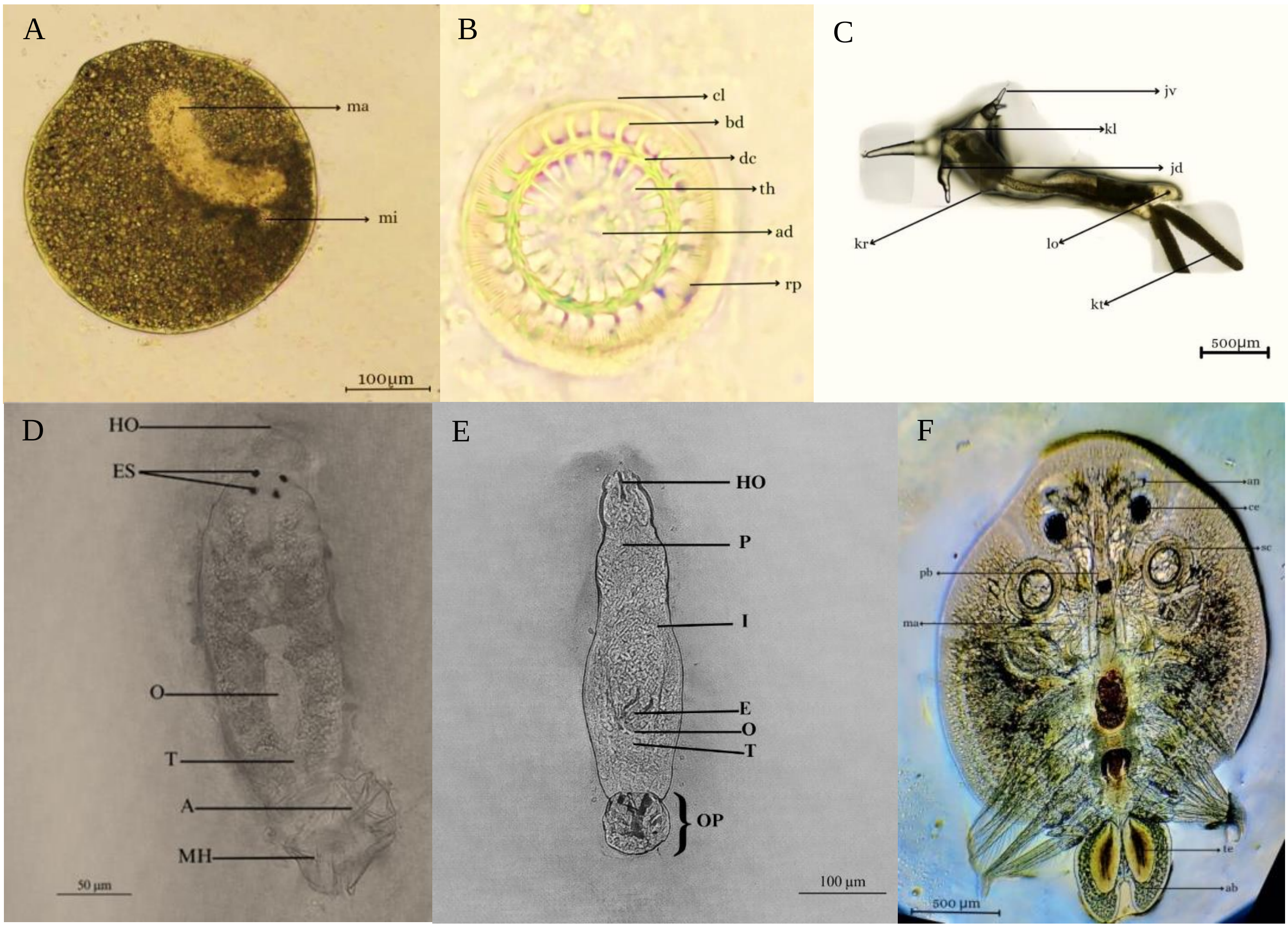
$$\text{Intensity} = \frac{\text{Number of parasite infecting the fish}}{\text{Number of fish infected with parasite}}$$

RESULTS

Parasite infestation

Four types of ectoparasites were found infesting *Puntius* fish, while five types of ectoparasites were found infesting *Anabas* fish cultured at the location of the study (Figure 1). Based on the results of the research, it was observed that *Anabas* fish were infested with protozoan parasites, arthropod parasites, copepod parasites, and monogenea parasites. No arthropod and copepod parasites were found to infest *Puntius* fish.

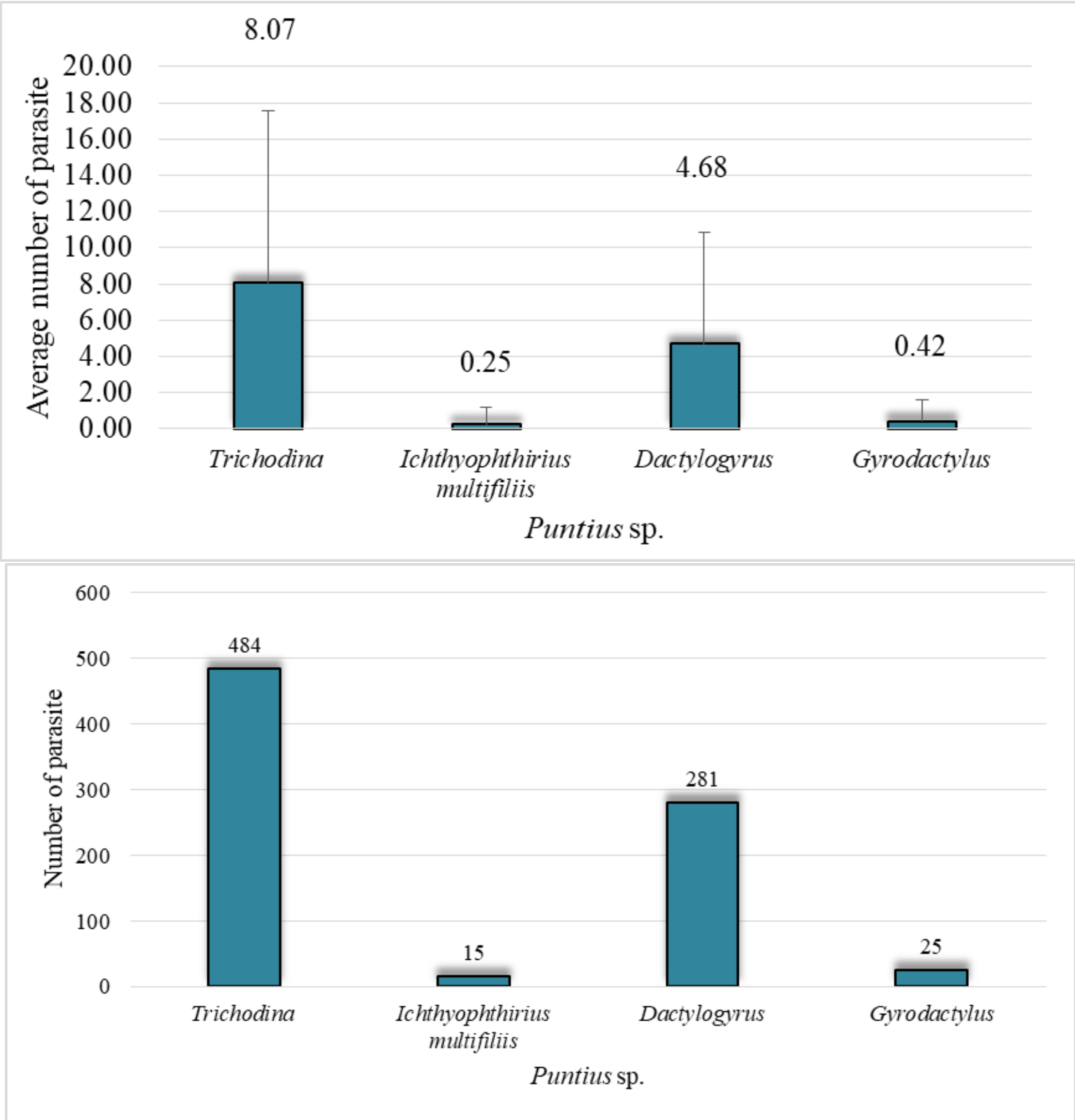
Figure 1. Ectoparasites found infested *Puntius* sp. and *Anabas* sp. A. *Ichthyophthirius multifiliis*, ma : macronucleus, mi : micronucleus; B. *Trichodina*, cl = cilia; bd = blade; dc = denticle; th = thorn; ad = adhesive disk; rp = radial pins; C : *Lernaea cyprinacea*, jv = ventral hook; kl = lobus head; jd = dorsal hook; kr = swim leg; lo = ovulation pore; kt = egg sac; D : *Dactylogyrus*, HO = Head organ ; ES. Eye spot ; O. Ovarium ; T. Testis ; A. Anchor ; MH. Marginal hooks; E : *Gyrodactylus*, HO = Head organ ; P. Pharynx ; I. Intestine ; E. Embrio; O. Ovarium ; T. Testis ; O. Opisthaptor ; F. *Argulus japonicus*, an = antenna; ce = compound eye; pb = probocis; ma = maxilla; su = sucker; te = testis; ab = abdomen.



Parasite Intensity

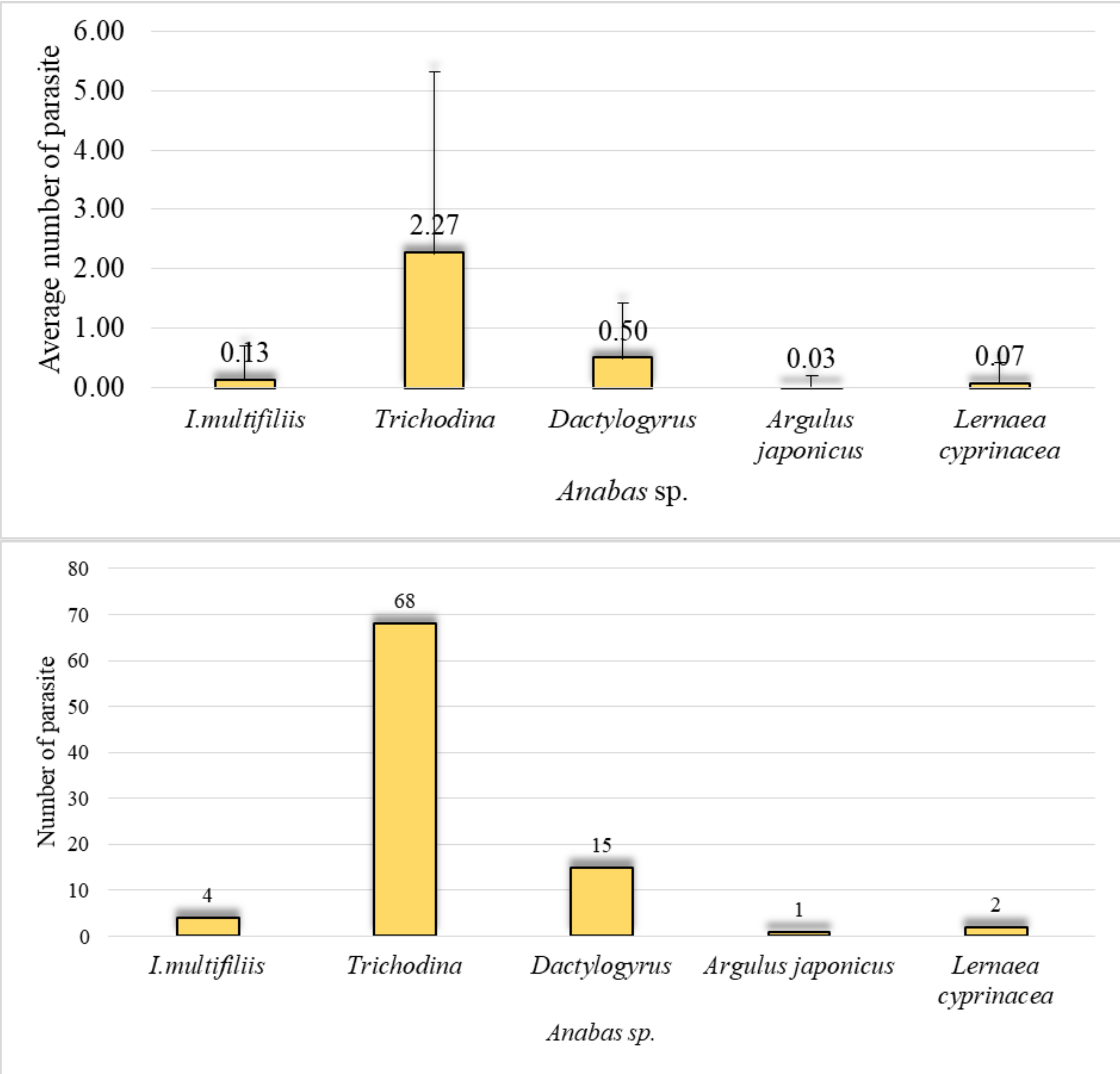
Based on the study shown in Figure 2, *Trichodina* was found as many as 484 individuals with mean number at 8.07. Another parasite found in large numbers was *Dactylogyrus* with 281 individuals at mean number of 4.68. The mean number of *Gyrodactylus* parasites was quite low at 0.42 with a total of 25 parasites and *Ichtyophthirius multifiliis* had a mean number of 0.25 with a total of 15 parasites. According to this calculation, the highest number of parasites found was *Trichodina*.

Figure 2. Parasite intensity in *Puntius* sp.



Likewise, from the Figure 3, *Anabas* fish were infested with *Trichodina* with a total of 68 parasites and an average number of 2.27, followed by *Dactylogyrus* which had an average number of 0.50 with a total of 15 parasites. The average number of other parasites was *I. multifiliis* at 0.13 with a total of 4 parasites and *Argulus japonicus* had an average of 0.03 with a total of 1 parasite. In *Anabas* fish, the parasite *Lernaea cyprinacea* was found to have an average of 0.07 with a total of 2 parasites in all samples. According to this calculation, it shows that in *Anabas*, the highest number of parasites was found to be *Trichodina*.

Figure 3. Parasite intensity in *Anabas* sp.



There are differences in the prevalence and intensity values of each ectoparasite infesting *Puntius* and *Anabas* fish cultured in Indonesia. Table 1 shows that *Trichodina* ectoparasites were found to have the highest prevalence values in *Anabas* and *Puntius* fish. On the other hand, the ectoparasite *Gyrodactylus* was found to have the lowest prevalence value in *Puntius* fish, and the ectoparasite *Lernaea cyprinace* was found to have the lowest prevalence value in *Anabas* fish.

These findings also indicate differences in the predilection behaviour of the ectoparasite Ichthyopthirius multifilis. Ichthyopthirius multifilis was found on the body surface and gills of Puntius fish, while Ichthyopthirius multifilis was found to infest only the body surface of Anabas fish. Trichodina was observed to infest Puntius and Anabas fish on the body surface and gills. Dactylogyrus was found only on the gills of Puntius and Anabas fish.

In addition to the intensity, there were differences in the intensity values of parasites in *Puntius* and *Anabas* fish. *Trichodina* has the highest intensity value in *Puntius* fish, while in *Anabas* fish, *Dactylogyrus* has the highest intensity. *Ichthyophthirius multifiliis* and *Argulus japonicus* were the lowest intensity parasites found in both endemic farmed fish.

Table 1. Ectoparasite infestation in *Puntius* sp. and *Anabas* sp. farmed in pond

Sample	Pond Type	Ectoparasite	Predilection	Prevalence (%)	Intensity (ind/fish)
Puntius sp. (n = 60)	Concrete pond	*Trichodina*	Body surface, gill	78.3	10.74
		Ichthyophthirius multifiliis	Body surface, gill	10	2.5
		Gyrodactylus	Body surface, gill	15	2.7
		Dactylogyrus	Gill	50	9.2
Anabas sp. (n = 60)	Concrete and earthen pond	*Ichthyopthirius multifilis*	Body surface	8.3	1.6
		Argulus japonicus	Body surface	5	1
		Lernaea cyprinacea	Body surface	1.7	2
		Trichodina	Fin, gill	61.7	4.05
		Dactylogyrus	Gill	45	1.86

Parasite distribution

The distribution of each type of parasite in *Puntius* fish and *Anabas* fish has the same pattern. Based on the results of boxplot analysis, outlier data were found in all types of parasites infesting *Puntius* and *Anabas* fish (Figures 4 and Figure 5). This indicates that there are still fish with a very high number of parasites or much higher than other fish in the same population. The wide shape of the blox indicates a high diversity of data, which can be interpreted that the number of parasites (individuals) in each fish is quite diverse.

Water quality

The result of water quality measurement is shown in Table 2.

Table 2. Water Quality Measurement

Parameter	*Puntius* pond	*Anabas* pond
Temperature (^{0}C)	27 -28.25	27 - 31
pH	7 - 8	7 - 8
Dissolved Oxygen (mg/l)	6.78 – 8.59	6.78 – 8.59

Water quality test results were used as supporting information for this study. Temperature, pH, DO are water quality parameters that need to be known in fish farming activities [27]. The water quality of the farming ponds in the locations was considered acceptable and optimal for Puntius and Anabas fish based on the measurement data of water quality parameters [28]. However, despite the optimal water quality, the farmed *Puntius* and *Anabas* fish still showed parasite infestation. These optimal water quality parameters can be a contributing factor to fish health, and on the contrary, a limiting factor for parasite development.

Figure 4. Boxplot Analysis of parasites from *Puntius* sp.

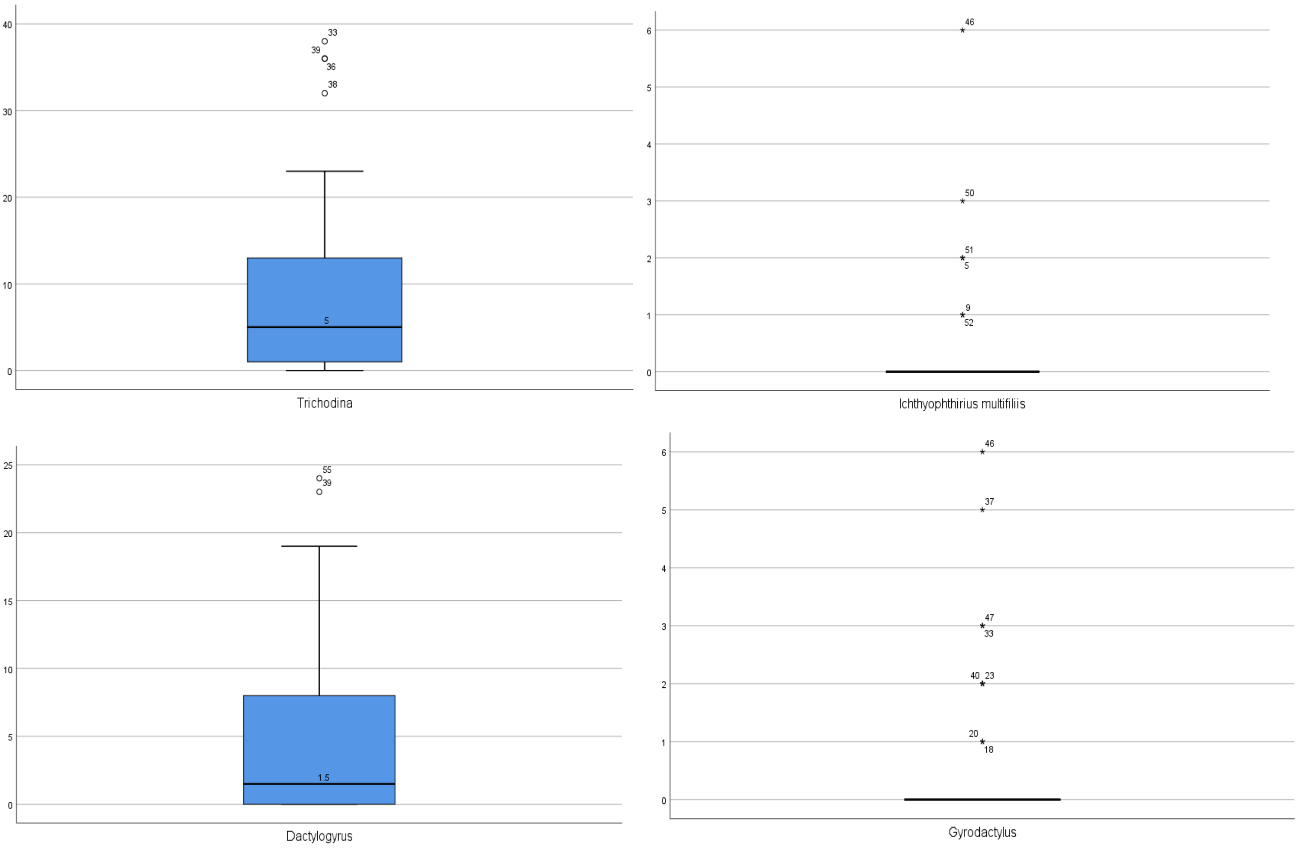
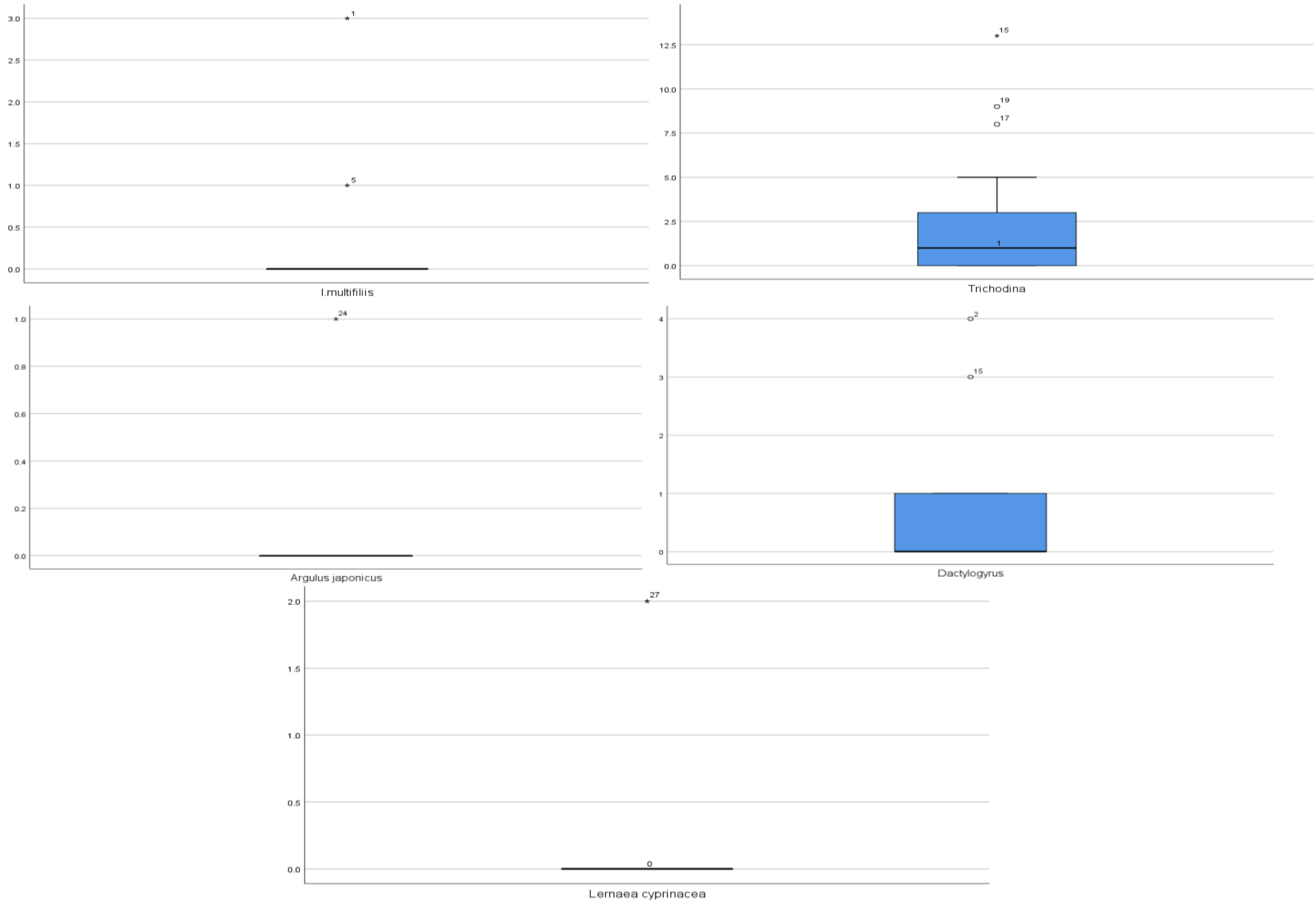


Figure 5. Boxplot Analysis of parasites from *Anabas* sp.



DISCUSSION

Ectoparasite infestation in fish can be the result of an imbalance between the environment, the parasite, and the fish as the host. A poor environmental condition, usually due to untreated aquaculture water that causes suboptimal water quality, will cause fish stress. Stressed fish have a low immune system and are easily infested with ectoparasites [10].

Many ectoparasites found in this study were found to infest the body surface and gills. Also, ectoparasites infest the body surface because this part is the outermost layer which contains mucus, epithelial tissue and blood vessels which are the site of parasite habitation [29]. This is in line with the opinion [30] which states that the fish body surface is directly related to the environment, making it easier for ectoparasite infestation to take part in because it contains a lot of mucus and epithelial tissue which is a suitable living space for ectoparasites and a food source. Fish mucus contains lysosyme, complement, and antibodies that play a role in degrading pathogens. Ectoparasites inhabit the gills because it is an essential organ in the respiratory process of fish and the exchange process between oxygen (O_2) and carbon dioxide (CO_2). Through the gill, oxygen will be absorbed by the blood capillaries in the gills. Then the secondary lamella diffusion processes occur and carbon dioxide is released into the water through the gills. In the gills there are many blood capillaries, where blood is a suitable source of nutrients for the proliferation of parasites in the gills [31].

Trichodina and *Dactylogyrus* were found to have the highest intensity values in both hosts. This could be due to the fact that the water source used was river water, and both endemic fish were cultured in stagnant water conditions. Aquaculture ponds with stagnant and non-flowing water have a greater potential for parasite infestation compared to ponds with fast-flowing water [32]. The high prevalence and intensity of *Trichodina* can be described by its rapid division ability, which is every 30 minutes, the suitability of pond temperature in the range of 20^0C-29^0C, and the lack of intermediate host requirements for *Trichodina* to multiply [33]. The transmission of *Trichodina* occurs through water or direct contact with infested fish and the transmission will be even faster if the water quality is low. *Trichodina* is found predominantly in freshwater fish farming. *Trichodina* grows well in shallow and stagnant ponds [34]. *Trichodina* is a passive opportunistic parasite that can easily infect fish, especially in pond conditions with low water quality.

The prevalence and intensity values of *Dactylogyrus*, *Gyrodactylus* and *Ichthyophthirius multifiliis* tend to be lower than *Trichodina*. *Dactylogyrus* undergoes rapid reproduction when the ambient temperature is high. *Dactylogyrus* is known as the gill fluke [35] and is commonly found in the gills according to our study. Meanwhile, *Trichodina* has a predilection for the entire body of the fish, including the body surface, fins, and gills [34]. Although the temperature was suitable for *Dactylogyrus* proliferation, the number of *Trichodina* parasites was also found in high numbers because *Trichodina* does not have a specific predilection site in comparison to *Dactylogyrus* which is only dominant in the gills. *Dactylogyrus* requires a breeding time of 27-28 days at 8°C, 10-15 days at 12°C, 3-5 days at 20°C and 1-4 days at 24-28°C [23]. The average egg hatching of *Dactylogyrus* depends on temperature, where at low temperatures it will require several months to hatch, while at high temperatures it will hatch in around 4 days. The optimal conditions for *Dactylogyrus* reproduction that occurred in the research pond at the time of sampling could be one of the contributing factors to the high value of *Dactylogyrus* intensity.

The rapid reproductive process of *Dactylogyrus* is in contrast to *Gyrodactylus*. *Gyrodactylus* reproduces viviparously, producing one individual at a time. *Gyrodactylus* is produced through a polyembryonic process that produces three or four embryos from a single egg (one inside another egg). The first or outermost embryo develops into a single individual in the maternal womb. Within the first uterus, a second embryo develops, and a third embryo develops within the second embryo and so on. The last- born *Gyrodactylus* has no embedded embryo so it starts the cycle from a fertilized egg and produces 3 or 4 embryos. The period of embryonic development from egg to mature individual is around 3 days (60 hours) [23], while according to [36] the process takes 4 - 5 days. The life cycle of *Gyrodactylus* is also reported to takes 9-15 days with an optimal temperature of around 120^0C [37]. These limiting factors could be the cause of the low prevalence and intensity of *Gyrodactylus* in this study.

Other ectoparasites found to infest *Anabas* sp. namely *Argulus* and *Lernaea* are parasites that are often reported to infest freshwater fish. Argulus infestation in *Anabas* fish has been reported previously by [38], [10], and [39] in wild populations. There have been no reports of *Lernaea* infestation in *Anabas* fish in either aquaculture or open water conditions. According to [40], differences in prevalence rates and intensity can be caused by environmental conditions in this context water quality, weather or season, fish density, and type of feed. In addition, the high prevalence and intensity values at each sampling location are influenced by internal and external factors. Internal factors can be in the form of poor body condition of fish that are susceptible to disease. External factors can be in the form of a decrease in water quality due to excessive feeding, cultivation activities or the condition of the research location which is flooded and there is no change of water which causes parasites to appear (10).

CONCLUSION

This study found that *Puntius* sp. and *Anabas* sp. are endemic fish that are potentially infested with parasites under culture conditions. This research is expected to be an anticipative step toward parasites that could potentially endanger the production of *Puntius* and *Anabas* fish. It also shows the concern for parasitosis that occurs in endemic farmed fish.

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Declaration of conflict of interest

The authors declare that there is no conflict of interest.

Ethical of approval

The following ethical approvals were obtained from Department of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, and instructions followed in the Laboratory Code of Animal Care were followed

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